

Claims

1. A method for the stabilisation of nucleic acid from a biological sample, which comprises:
 - (a) collecting a biological sample;
 - (b) treating the sample so that a proportion of the 2', 3' or 5'-OH positions of the nucleic acid are modified with a protecting group; and
 - (c) subjecting the treated sample to one or more steps to isolate nucleic acid therefrom; wherein the modified nucleic acid is subjected to a deprotection step comprising treatment with a primary amine to remove the protecting group.
2. A method according to claim 1, wherein the biological sample comprises viruses, cells, body fluids, blood, serum or plasma.
3. A method according to claim 1 or claim 2, wherein the biological sample comprises a clinical sample or a human pathogen.
4. A method according to any of claims 1 to 3, wherein the nucleic acid is single or double stranded RNA or DNA.
5. A method according to claim 4, wherein the sample is treated with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA.
6. A method according to any preceding claim, wherein step (b) is carried out in the presence of an organic solvent.
7. A method according to claim 6, wherein the organic solvent has a flashpoint above 37°C.
8. A method according to claim 6 or claim 7, wherein the organic solvent is capable of forming a homogeneous solution with human blood when mixed in a ratio of 5:1 (vol:vol).

9. A method according to any preceding claim, wherein the primary amine is ethylenediamine, diethylenetriamine, triethylenetetramine, lysine or arginine.
10. A method according to any preceding claim, wherein step (c) comprises:
 - (i) binding the nucleic acid to a solid phase;
 - (ii) optionally washing the solid phase to remove contaminants; and
 - (iii) optionally eluting the nucleic acid from the solid phase.
11. A method according to claim 10, wherein the solid phase comprises magnetic particles.
12. A method according to claim 10 or claim 11, wherein the solid phase contains a metal or metal ion capable of coordinating with phosphate.
13. A method according to claim 12, wherein the nucleic acid is eluted with a chelator.
14. A method according to claim 13, wherein the chelator is EGTA and elution is carried out at a pH above 9.
15. A method according to claim 13, wherein the chelator is a salt of ammonia or tetraalkylammonium.
16. A method according to claim 13, which further comprises removing the chelator from the nucleic acid by ultrafiltration, photosensitivity of the chelator or affinity purification using an affinity tag on the chelator.
17. A method according to any of claims 12 to 16, wherein the solid phase comprises hydroxylapatite.
18. A method according to claim 17, wherein the hydroxylapatite is pretreated with a phosphate-containing compound.

19. A method according to claim 17 or claim 18, wherein the hydroxylapatite is washed in step (ii) with an amine.
20. A method according to claim 19, wherein the amine is a primary amine.
21. A method according to claim 20, wherein the deprotection step comprises step (ii).
22. A method according to any of claims 9 to 18, wherein the deprotection step occurs between step (i) and step (ii).
23. A method according to claim 10 or claim 11, wherein the solid phase comprises silica.
24. A method according to claim 10 or claim 11, wherein the solid phase has immobilised thereon nucleic acid complementary to the nucleic acid targeted for isolation.
25. A method according to claim 24, wherein the nucleic acid targeted for isolation is RNA, which is subjected to the deprotection step prior to binding to the solid phase.
26. A kit for use in a method for the stabilisation of nucleic acid from a biological sample; which comprises:
 - (i) a reaction system for treating the sample so that a proportion of the 2', 3' or 5'-OH positions of the nucleic acid are modified with a protecting group;
 - (ii) an isolation system for subjecting the treated sample to one or more steps to isolate nucleic acid therefrom; and
 - (iii) a primary amine for subjecting the modified nucleic acid to a deprotection step to remove the protecting group.
27. A kit according to claim 26, wherein the reaction system comprises a reactant capable of covalently modifying the 2'-OH position of the ribose rings of RNA.
28. A kit according to claim 26 or claim 27, wherein the reaction system includes an organic solvent.

29. A kit according to claim 28, wherein the organic solvent has a flashpoint above 37°C.
30. A kit according to claim 28 or claim 29, wherein the organic solvent is capable of forming a homogeneous solution with human blood when mixed in a ratio of 5:1 (vol:vol).
31. A kit according to any of claims 26 to 30, wherein the primary amine is ethylenediamine, diethylenetriamine, triethylenetetramine, lysine or arginine.
32. A kit according to any of claims 26 to 31, wherein the isolation system comprises:
 - (a) a solid phase for binding the nucleic acid;
 - (b) optionally a washing solution for washing the solid phase to remove contaminants; and
 - (c) optionally an elution solution for eluting the nucleic acid from the solid phase.
33. A kit according to claim 29, wherein the solid phase comprises magnetic particles.
34. A kit according to claim 32 or claim 33, wherein the solid phase contains a metal or metal ion capable of coordinating with phosphate.
35. A kit according to claim 34, wherein the elution solution comprises a chelator.
36. A kit according to claim 35, wherein the chelator is EGTA.
37. A kit according to claim 35, wherein the chelator is a salt of ammonia or tetra-alkylammonium.
38. A kit according to claim 35, wherein the chelator is a photosensitive chelator or has an affinity tag.
39. A kit according to any of claims 34 to 38, wherein the solid phase comprises hydroxylapatite.

40. A kit according to claim 39, wherein the hydroxylapatite is pretreated with a phosphate-containing compound.
41. A kit according to claim 39 or claim 40, wherein the washing solution comprises an amine.
42. A kit according to claim 41, wherein the amine is the primary amine.
43. A kit according to claim 32 or claim 33, wherein the solid phase comprises silica.
44. A kit according to claim 32 or 33, wherein the solid phase has immobilised thereon nucleic acid complementary to the nucleic acid targeted for isolation.